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Jeremy Tiemann, Jordan Hartman, Josh Sherwood, Philip Willink, and Eric Larson

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Authors: Jeremy Tiemann[1], Jordan Hartman[2], Josh Sherwood[1], Philip Willink[3], and Eric Larson[2]

[1] Illinois Natural History Survey, Prairie Research Institute, University of Illinois, Champaign-Urbana
[2] University of Illinois, Champaign-Urbana
[3] Field Museum, Chicago

Executive Summary

Recent investigations into the rapid expansion of Banded Killifish (*Fundulus diaphanus*) in Illinois has brought to light significant conservation implications. Previous data suggested that Illinois should be inhabited by the Western subspecies of Banded Killifish (*F. d. menona*), though morphological data from recently encountered populations reveals some populations might be comprised of the Eastern subspecies (*F. d. diaphanus*) and/or subspecies hybrids. Until now, the Illinois Endangered Species Protection Board has not had to recognize subspecies, and it was possible the recent expansion of Banded Killifish populations throughout Illinois could have led to the removal of its threatened status, regardless of the subspecies responsible for the expansion. The goal of our project was to investigate the genetic composition of expanding populations of Banded Killifish across Illinois, with an emphasis on determining whether this recovery of a rare taxa is of the native Western subspecies, an invasion of the non-native Eastern subspecies, or whether hybridization between subspecies is occurring.

We compared the molecular characteristics of Banded Killifish populations in Illinois and surrounding areas to those from within the native ranges of the Western Banded Killifish and Eastern Banded Killifish. We collected Banded Killifish between 2017 and 2020 from nearly 100 sites around the Midwest – kettle lakes, southern Lake Michigan basin, the Chicago Area Waterway System (CAWS), upper Illinois River, lower Rock River, and Mississippi River – as well as areas representing known Western (e.g., Iowa, Minnesota) and Eastern (e.g., Maryland, Canada) subspecies. Genomic material was extracted from muscle tissues via common extraction kits and protocols and included the mitochondrial D-loop and nuclear X-*src* intron.

Our results show that there are three conservation management units within Illinois – 1) the kettle lake region of McHenry and Lake counties, which is the only area not influenced by the non-native Eastern Banded Killifish; 2) the Lake Michigan – CAWS – Illinois River (including Wolf Lake and Powderhorn Lake in Cook County) population that is predominately made up of the Eastern subspecies and hybrids; and 3) the Mississippi River (including lower Rock River) population, which contains both subspecies as well as subspecies hybrids. Our data should provide the resource managers of Illinois a complete understanding of the expansion of Banded Killifish in Illinois and provide a crucial step in conserving the native Western Banded Killifish, while recognizing the existence of a non-native taxa.

Cover photo: Western Banded Killifish (*Fundulus diaphanus menona*) x Eastern Banded Killifish (*Fundulus diaphanus diaphanus*) hybrid (P. Willink, photo).

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Introduction

The Banded Killifish *Fundulus diaphanus* (Figure 1; Figure 2) is a relatively widespread cyprinodontid species. Its range extends along the Atlantic coast from North Carolina to Nova Scotia and New Brunswick, the Great Lakes and St. Lawrence basins, and upper Midwestern states in the Mississippi River drainage (Page and Burr 2011). Two subspecies of Banded Killifish are recognized, and recent research suggests limited geographic overlap between the ranges of the two subspecies (April and Turgeon 2006). The Eastern Banded Killifish (*F. d. diaphanus*) occupies the Atlantic coast, inland to Lakes Ontario and Erie, whereas the Western Banded Killifish (*F. d. menona*) ranges from Lake Erie, west to Lakes Huron and Michigan, and the upper Mississippi River drainage. Range-wide populations of Banded Killifish are considered secure, but populations in Wisconsin and North Dakota are considered Vulnerable, West Virginia and Iowa are Imperiled, and Manitoba, Newfoundland, South Dakota, South Carolina, and Illinois are Critically Imperiled (NatureServe 2017).

Historical Illinois populations of the Western Banded Killifish were predominantly located in northeastern Illinois (Figure 3), where the fish could be observed schooling at the surface of the water in quiet, sheltered areas of clear, well-vegetated glacial lakes, coastal wetlands, drowned river mouths, and slow-moving streams (Smith 1979). Early accounts reported the killifish in the Fox and Des Plaines river drainages, coastal wetlands along Lake Michigan, and in a small area in the Salt Creek basin (Sangamon River drainage) in McLean County (Jordan 1877, 1878; Meek and Hildebrand 1910; Forbes and Richardson 1919, 1920; Willink 2017). Later, as lakes and wetlands were filled, streams were channelized and dredged, and multiple stressors deteriorated water bodies, the range of Banded Killifish in Illinois shrank (Figure 3), including the disappearance of the McLean County population, until the species was only found in a small number of glacial lakes in Lake and McHenry counties (Smith 1965, 1971, 1979). The Banded Killifish was listed as threatened in Illinois in 1989 due to restricted habitats or low populations in the state and was classified as such at the beginning of our project in 2017 (Illinois Endangered Species Protection Board 2015).

Since 2000, the Banded Killifish has become more prevalent and widespread in Illinois (Figure 3). Samples from Lake Michigan beach surveys first collected Banded Killifish in 2001 and the fish has subsequently become more widespread and abundant throughout Illinois (Willink et al. 2018). Moving inland, Banded Killifish became widespread in the Chicago Area Waterway System (CAWS) and upper Illinois River starting in 2006, including as far south as Peoria County (Tiemann et al. 2015; Willink 2017; Willink et al. 2018). Consequently, the Banded Killifish was discovered in the Mississippi River in Davenport, Iowa in 2009, and since has spread downstream as far as West Alton, Missouri (= Madison County, Illinois), in addition to moving into the Rock River basin upstream as far as Prophetstown, Whiteside County (Rivera et al. 2013; Tiemann et al. 2015; Hrabik 2016; Lamer et al. 2016; Willink et al. 2018, 2019).

While the sudden and drastic increase of a threatened species is often considered a conservation success story, recent morphological analyses of the “new” populations of Banded Killifish in Illinois suggested that those recent increases were not the Western Banded Killifish (Willink et al. 2018). Lateral scale rows of Banded Killifish collected in the Calumet Region appeared to be intermediate between the accepted counts of the Western and Eastern subspecies, suggesting that hybridization could be occurring in Illinois waters and the potential for populations of non-native Eastern subspecies in the state (Willink et al. 2018). April and

Turgeon (2006) noted natural hybridization of the subspecies in Lake Erie, as it is where the two subspecies potentially co-occur, yet all Banded Killifish in Illinois were hypothesized to be of the Western subspecies. Genetic analyses of the expanding Banded Killifish populations in Illinois were needed to determine whether recent expansions are due to increases in the native subspecies or an "invasion" of the non-native subspecies. However, because prior specimens examined were preserved in formalin, a genetic analysis could not be made. These analyses are a crucial step in conserving the Western Banded Killifish in Illinois and would provide managers further data in their attempts to maintain this threatened species.

Needs Assessment

This drastic range expansion phenomenon was especially alarming because it appeared to be the result of the introductions of the non-native Eastern subspecies or its hybridization with the native Western subspecies. Perhaps persisting mostly as hybrids of the two subspecies, the Banded Killifish is now more abundant than in either of the previous surveys of the state (Willink, et al. 2018, 2019). However, the Illinois Endangered Species Protection Board did not recognize Banded Killifish subspecies prior to our work (IESPB 2015). The recent apparent range expansions of Banded Killifish in Illinois could have resulted in the suggested removal of this species from the list of Endangered and Threatened Species in Illinois, possibly without considering which subspecies has expanded in the state, nor which subspecies is native. Conservation of the appropriate subspecies of Banded Killifish in Illinois would support the range-wide diversity of the species. Regardless of which subspecies is found to be the cause of the recent Banded Killifish expansion in Illinois, a genetic analysis of the current population would aid in the conservation of the species in the state. Populations of species with once limited ranges are susceptible to reduced genetic diversity from founder effects or population bottlenecks (Meffe 1986). Reduced genetic diversity can hinder a species' ability to adapt to changes in the environment or lead to the fixation of detrimental alleles (Meffe 1986), both of which could have negative effects on Banded Killifish populations in Illinois.

The objective of our study was to compare the molecular characteristics of Banded Killifish populations in Illinois to those from within the native ranges of the Western Banded Killifish (*F. d. menona*) and Eastern Banded Killifish (*F. d. diaphanus*). Analyses of these data would determine whether individual populations of Banded Killifish in Illinois are comprised of the native Western subspecies, the non-native Eastern subspecies, or potentially hybrids between the two. Because the Western subspecies is thought to be native to Illinois, identifying populations (if any) of the Eastern subspecies within the state would allow managers and decision makers to focus conservation efforts appropriately. For example, genetic analysis of the Mississippi River population would aid in determining whether this population is due to natural migrations, or from human error, and would allow managers to act accordingly. Genetic biogeographic patterns could reveal the pathway by which the Eastern subspecies spread, thus potentially identifying pathways that other invasive species could use in the future.

Methods

Sample Collection: We collected fishes at 66 sites from throughout Illinois and neighboring border counties in Indiana and Wisconsin, including kettle lakes, southern Lake Michigan basin, the CAWS, upper Illinois River, lower Rock River, and Mississippi River between 2017 and 2020 (Table 1; Table 2). Colleagues provided samples from areas representing known Western (e.g., Iowa, Minnesota) and Eastern (e.g., Maryland, North Carolina) subspecies. We targeted ten individuals from each site, and fish were collected using standard fisheries techniques and include seining, electrofishing, trapping via mini-fyke nets, and dip-netting. No efforts were made to determine relative abundance at a site, but we did make note of those sites where we failed to collect Banded Killifish (Table 2).

Complete individuals were anesthetized with methanesulfonate (MS-222), then euthanized and preserved in 95% EtOH, which allowed us to not only take tissue samples for genetic analyses, but also allowed us to conduct morphological analyses (e.g., scale counts) if evidence of genetic hybrids were discovered. Specimens are deposited in the Illinois Natural History Survey Fish Collection, Champaign, and are available for future studies.

Genetic Analyses: Genomic material were extracted from muscle tissues via common extraction kits and protocols (Clement 2002; Leigh 2015). Target loci for our analysis mirrored those described by April and Turgeon (2006), which consisted of the mitochondrial (mtDNA) D-loop and the nuclear X-*src* intron. Because the nuclear X-*src* intron was largely uninformative or invariable for our study specimens, consistent with April and Turgeon (2006) for our study region, results here focus exclusively on the mtDNA D-loop. D-loop primers are available in Lee et al. (1995). Extracted genomic materials were amplified via polymerase chain reaction (PCR), verified via gel electrophoresis, cleaned using standard ExoSap-IT protocols, and sequenced. Sequencing of amplified materials were performed by the University of Illinois W.M. Keck Center. A mtDNA haplotype network was constructed and visualized using PopArt (http://popart.otago.ac.nz/index.shtml) and Banded Killifish individuals were assigned to Western or Eastern subspecies by mtDNA loop consistent with April and Turgeon (2006).

Results

We collected or secured Banded Killifish at 30 sites from throughout the Midwest (Table 1; Table 3). Based upon genetic analysis, our haplotype data identified three genetically distinct groups within Illinois (Figure 4):

1) The kettle lake region of McHenry and Lake counties (including the upper Des Plaines River), where we found 100% of the mtDNA haplotypes belonged to the Western subspecies (Table 3; Figure 4). There was no evidence that the non-native Eastern subspecies has invaded and polluted the native stock of the Western subspecies in this region.
2) The Lake Michigan – CAWS – Illinois River population (including Wolf Lake and Powderhorn Lake and lower Des Plaines River in Cook County) that is predominantly made up of the non-native Eastern Banded Killifish and/or hybrids (Table 3; Figure 4). This region varied from 100% of the mtDNA haplotypes belonging to the Eastern subspecies (e.g., Lake Michigan) to a 50%-50% split between the Eastern subspecies and

native Western subspecies (e.g., Wolf Lake). The lone exception is Powderhorn Lake, which appears to be exclusively the native Western Banded Killifish.

3) The Mississippi River population (including lower Rock River), which contains both the Western Banded Killifish and Eastern Banded Killifish. This population is not robust as those in other areas as the fish was found in only a few sites and in low numbers (Table 1; Table 2). This region varied from 33% to 66% of the mtDNA haplotypes belonging to the native Western subspecies (Table 3; Figure 4).

Discussion

Within Illinois, Banded Killifish have become established in the CAWS, upper Illinois River, lower Rock River basin, and the Mississippi River as far downstream as the greater St. Louis metroplex, as well as continuing to persist in kettle lakes in McHenry and Lake counties (Tiemann et al. 2015; Willink et al. 2018, 2019). The fish has become more prevalent and widespread in the southern Lake Michigan drainage since the turn of the century (Willink et al. 2018, 2019). For example, samples from Lake Michigan beaches, as well as the St. Joseph River in Michigan, first recorded Banded Killifish within the last 20 years, and the fish has drastically expanded its range since. Morphological (Willink et al. 2018) and now genetic data (Table 3) show the non-native Eastern subspecies has invaded and is largely responsible for this explosive range expansion in those areas outside of the kettle lake region.

There appears to be at least three conservation management units, each with its unique history, habitat requirements, and potential future trajectories. The first is the kettle lake population in McHenry and Lake counties (including the upper Des Plaines River). Because this area is 100% of the mtDNA haplotypes belonging to the Western subspecies (Table 3), there is no indication that the rapid expansion occurring elsewhere in the state has reached this region along the Wisconsin border. Expansion here would require the upstream movement of fish past dams in the Fox and Des Plaines rivers, as well as their tributaries. Several studies have shown that dams, even lowhead dams, can alter the fish assemblage and limit the upstream migration of fishes (Watters 1996; Tiemann et al. 2004; Santucci et al. 2005; Tiemann et al. 2007; Slawski et al. 2008; Butler and Wahl 2010). Additionally, the connections that occurred between glacial lakes and streams of this area have been disrupted due to anthropogenic / urbanization disturbances, including extensive residential development of shorelines, high nutrient and siltation loads, and removal of aquatic vegetation, (Smith 1965, 1971). For the time being, this area appears to be isolated from those areas harboring the non-native Eastern subspecies and likely has one of the last remaining populations of native Banded Killifish in the state. The Illinois Department of Natural Resources with input from the Illinois Endangered Species Protection Board could re-evaluate the conservation status/risk of extirpation of this population, but interpretation of how the population should remain with the Illinois Endangered Species Protection Board and should be protected by the IDNR and IESPB as such.

It should be noted the Banded Killifish was one of several rare fishes authorized by the Illinois Department of Natural Resources for introduction as a refuge population in the Prairie Crossing Sanctuary Pond in 1998 (see Schaeffer et al. 2012; Bland 2013). The Sanctuary Pond is a ~3-acre retention pond located in the upper Des Plaines River drainage just north of Libertyville in Lake County. The Banded Killifish thrived and reproduced and could have

provided source populations that led to subsequent downstream emigration and recolonization of sites within their historic range within the upper Des Plaines River basin (Tiemann 2017), but it seems highly unlikely this population was responsible for the drastic range expansion, especially when considering the brood for the project came from the kettle lake region of northern Illinois (Schaeffer et al. 2012) and were the native Western subspecies (Table 3).

The second conservation management unit is the Lake Michigan – CAWS – Illinois River population (including Wolf Lake and Powderhorn Lake in Cook County, as well as the Snake Den Hallow refugee site in Knox County) that is dominated by the Eastern mtDNA haplotypes (Table 3). This population should be debated whether to consider it non-native. Based upon its current trajectory (i.e., Willink et al. 2018), this population appears to be following a similar expansion trajectory of the Round Goby (*Neogobius melanostomus*), Zebra Mussel (*Dreissena polymorpha*), and other non-native species of Lake Michigan and expand unabated downstream, as well as slowly expand upstream in several streams, like the Des Plaines River, in the Chicagoland region (Mills et al. 1993; Irons et al. 2006; Irons et al. 2009; Jacobs and Keller 2017). Specifically, the Des Plaines River should be broken up in two management units based upon the mtDNA haplotypes (Table 3) – the upper Des Plaines, which is part of the kettle lake region in Lake County and contains the Western subspecies, and the lower Des Plaines in Cook County and contains the Eastern subspecies. Our data suggest these two populations are still separated by nearly 80 km but should continue to be monitored to determine if the populations are converging.

The third conservation management unit is Mississippi River population (including lower Rock River) that continues to expand on the western side of Illinois (Figure 3). This population is a perplexing one, as there are no historical records for Banded Killifish near the Illinois portion of the river until 2009, when the species appeared in a tributary near Davenport, Iowa (Willink et al. 2019), and subsequently appeared in a rearing pond at the Fairport State Fish Hatchery near Muscatine, Iowa (Schmidt 2016). Our 2017-2020 specimens were identified as both the Western and Eastern subspecies (Table 3). The appearance of this population, at least the Western subspecies, could be explained by either downstream migration from Mississippi River populations in Wisconsin and Minnesota or from those in the Iowa and Des Moines rivers in Iowa (all of which are 150+ km away from the Illinois records). Human-mediated release (e.g., improper disposal of bait bucket fish) also could introduce both the Western and Eastern subspecies to new locations simultaneously if source populations for these fish contained both subspecies (i.e., contemporary Wolf Lake). While it is possible the fish migrated from populations in the Illinois River through the Hennepin Canal, it seems improbable given the number of dams (>25) that exist in the now defunct canal. Regardless, the fish is now found from the Quad Cities (Pool 15) to the greater St Louis metroplex (Pool 26) but appears to occur in small and isolated populations.

While we could not identify the exact invasion route into northeastern Illinois (e.g., CAWS, upper Illinois), an interesting observation made by Smith and Harris (2020) cannot be ignored. They collected Banded Killifish about 3 km from shore and in depths up to nearly 10 m in the open waters of Lake Michigan. While the authors did not identify subspecies, their capture shows the killifish using habitats farther from shore and in deeper waters than had been previously reported. Smith and Harris (2020) also stated that Banded Killifish does not recruit well to traditional fishing gear, especially those used in large bodies of water. This

observation, coupled with the fact that the Eastern subspecies is known from Lake Erie (Trautman 1981; April and Turgeon 2006), could imply that the non-native Eastern subspecies has spread and gone largely undetected throughout the Laurentian Great Lakes, and only recently has invaded Illinois' inland waters through the CAWS, which includes the Chicago Sanitary and Ship Canal. The CAWS is the only connection between the Great Lakes and Mississippi River ecosystems that maintains continuous aquatic habitat and has been implicated in the dispersal of several non-native aquatic species (Jacobs and Keller 2017).

An interesting phenomenon is occurring in Wolf Lake in the Calumet Region in Cook County. Known populations of Banded Killifish have persisted in this modified wetland complex over time (Meek and Hildebrand 1910; Forbes and Richardson 1920; Retzer and Batten 2005; Willink 2009). However, the Eastern subspecies has invaded Wolf Lake and is now sympatric with the resident Western subspecies population (Table 3). This invasion is likely due Wolf Lake's connections with Lake Michigan via the Calumet River (Greenberg 2002; Willink 2009). Hybridization is possible at locations where both subspecies were found together, like Wolf Lake, but this theory needs to be investigated by more resolved nuclear genome data than the uninformative X-src intron we attempted in our study. However, we did have some specimens from Wolf Lake that had mismatched X-src and D-loop data and would suggest hybridization. While not directly connected, Wolf Lake and Powderhorn Lake can join during extreme flooding, thus allowing for fish movement between the two lakes. It will be interesting to monitor the Powderhorn sub-population over time.

An important consideration when considering future dispersal of the Eastern Banded Killifish is that Trautman (1981) reported that the Eastern subspecies is more tolerant of pollution and not as reliant on vegetation for spawning, suggesting it might be more adaptable to highly altered areas (e.g., CAWS, upper Illinois River) than the Western subspecies. Also, the native range of Eastern subspecies extends farther south than the Western subspecies, suggesting increasing water temperatures due to climate change also could potentially benefit Eastern Banded Killifish or their hybrids (Willink et al. 2018, 2019).

Management implications and applications

Our data should provide the natural resource managers of Illinois a complete understanding of the expansion of Banded Killifish in Illinois and provide a crucial step in conserving the native Western Banded Killifish, while recognizing the existence of a non-native taxa. Data from this project were used to evaluate the status of Banded Killifish in Illinois. Based upon the analysis of molecular data, we determined the only areas with exclusively the native Western Banded Killifish haplotype are the kettle lake region in McHenry and Lake counties and Powderhorn Lake in Cook County. These areas historically supported and continue to support a robust, albeit small and fragmented, population of the native subspecies (Forbes and Richardson 1920; Smith 1979; Willink et al. 2018), which in our opinion, still warrants recognition as state-threated by the Endangered Species Protection Board of Illinois and was recently recognized as such (IESPB 2020). Even though there are Western Banded Killifish haplotypes elsewhere, they are sympatric with non-native Eastern Banded Killifish and may not persist long-term due to displacement by hybridization and introgression (i.e., Huxel 1999; Perry et al. 2002). Therefore, we believe all other areas should be considered lost-native

populations (e.g., Wolf Lake) or not native to Illinois (e.g., Lake Michigan proper, CAWS, upper Illinois River) and therefore do no warrant protection.

Our analyses are a crucial step in conserving the Western Banded Killifish and will provide natural resource managers further data in their attempts to maintain this rare species and develop conservation plans. Our results can now be used to define conservation management units. Additionally, although the emphasis of our project was Illinois, Banded Killifish distributions extend into neighboring states, indicating that any management implications in Illinois could have a bearing on the conservation of the species in Indiana, Wisconsin, Iowa, Missouri, and beyond. It is possible that other states are in a similar position but have not examined the genetic composition of their Banded Killifish populations. We strongly suspect this is the case in Indiana, Wisconsin, and Michigan, as areas along Lake Michigan appear to have been invaded by the non-native Eastern subspecies but natural resource agencies might not yet to recognize subspecies. Lastly, a population of Eastern Banded Killifish in the Ohio River (see Trautman 1981 and Page and Burr 2011) is slowly migrating downstream, and has been reported as far as Greenup County, Kentucky near the Sandy River – Ohio River confluence (Kentucky Afield 2020). If this population ever reached Illinois, it should be recognized as a non-native population.

It is unknown what, if any, the potential ecological effects the invasion of the Eastern Banded Killifish or their hybrids will have, especially in areas where Banded Killifish had not been found in the past. For example, further expansion throughout the Illinois and Mississippi river drainages could potentially lead to “unnatural” competitive pressures on species that have not encountered the Banded Killifish before. Close monitoring of the expansion is needed.

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Table 1 – List of locations where we collected Banded Killifish in Illinois, Indiana, and Wisconsin during our 2017-2020 study.

State	County	Stream / Lake	Basin	Lat / Long	Common location
IL	Lake	Bangs Lake	(Fox River Dr.)	42.2689, -88.1375	Wauconda
IL	Lake	Lake Zurich rentention pond	(Fox River Dr.)	42.2037, -88.1101	Lake Zurich retention pond
IL	Lake	Loon Lake	(Fox River Dr.)	42.4511, -88.0783	2 mi SSE Antioch
WI	Kenosha	Cross Lake	(Fox River Dr.)	42.4983, -88.0907	1 mi N Antioch, IL
WI	Walworth	Lake Geneva	(Fox River Dr.)	42.5594, -88.4667	Lake Geneva, south shore, 1/2 mi E 'The Narrows' adjacent to golf course
IL	Will	Des Plaines River	(Illinois River Dr.)	41.5107, -88.0925	Brandon Road Pool, near Joliet
IL	Cook	Chicago Sanitary & Ship Canal	(Lake Michigan - Illinois River Dr.)	41.7927, -87.8168	Summit, I-55 bridge
IL	Cook	North Branch Chicago River	(Lake Michigan - Illinois River Dr.)	41.9160, -87.6619	Chicago, Cortland Ave Bridge
IL	Will	Chicago Sanitary & Ship Canal	(Lake Michigan - Illinois River Dr.)	41.5906, -88.0674	Lockport Pool, near Lockport
IL	Cook	Lake Michigan	(Lake Michigan Dr.)	41.7762, -87.5718	Chicago, Jackson Park Outer Harbor
IL	Cook	Lake Michigan	(Lake Michigan Dr.)	41.9674, -87.6407	Chicago, Montrose Beach, backwater pool next to dog beach
IL	Cook	Wolf Lake - center-west pond	(Lake Michigan Dr.)	41.6691, -87.5359	3.5 mi N Calumet City, south of the boat launch
IL	Cook	Wolf Lake - northeast pond	(Lake Michigan Dr.)	41.6620, -87.5253	3.5 mi N Calumet City, at end of road that straddles the state-line
IL	Cook	Wolf Lake - southwest pond	(Lake Michigan Dr.)	41.6642, -87.5363	3.5 mi N Calumet City, near the outfall of Indian Creek
IL	Cook	Powderhorn Lake	(Lake Michigan Dr.)	41.6397, -87.5332	1.5 mi N Calumet City, near boat launch
IL	Lake	Lake Michigan	(Lake Michigan Dr.)	42.4924, -87.8030	North Point Harbor Beach, Winthrop Harbor
IL	Lake	Lake Michigan	(Lake Michigan Dr.)	42.3645, -87.8139	Waukegan, North Shore Beach, among the vegetation
IN	Lake	Lake Michigan	(Lake Michigan Dr.)	41.6199, -87.2696	Gary, Marquette Park Beach, at boat ramp
IN	Lake	Wolf Lake	(Lake Michigan Dr.)	41.6682, -87.5116	Whiting, Wolf Lake Park, along parking lot shore line
WI	Racine	Lake Michigan	(Lake Michigan Dr.)	42.7467, -87.7815	Racine, Racine Zoo Beach, backwater area
WI	Racine	Root River	(Lake Michigan Dr.)	42.7257, -87.7958	Racine, along shore just west of the 6th Street Bridge
IL	Grundy	Illinois River	(Mississippi River Dr.)	41.4016, -88.2800	Dresden Pool, near Dresden
IL	Grundy	Illinois River	(Mississippi River Dr.)	41.3203, -88.7005	Marseilles, Mallard Bay boat launch [Marseilles Pool]
IL	Hancock	Mississippi River	(Mississippi River Dr.)	40.4085, -91.3526	Pool 19, at confluence with Chaney Creek, Hamilton
IL	Knox	pond - Snake Den Hollow	(Mississippi River Dr.)	41.0240, -90.0810	Broodstock source: Marshall State Fish & Wildlife Area, delta of Crow Creek
IL	La Salle	Illinois River	(Mississippi River Dr.)	41.3269, -89.0061	boat launch in Starved Rock State Park, opposite Uttica [Peoria Pool]
IL	Rock Island	Rock River	(Mississippi River Dr.)	41.4621, -90.4984	Milan, downstream of I-74 at Harold's Landing
IL	Whiteside	Rock River	(Mississippi River Dr.)	41.6726, -89.9316	Prophetstown, at boat launch
IL	Lake	Des Plaines River	(upper Des Plaines River Dr.)	42.3053, -87.9547	Libertyville, IL Rte 137, Independence Grove Forest Preserve
IL	Lake	Sanctuary Pond	(upper Des Plaines River Dr.)	42.3338, -88.0135	Grayslake, Prairie Crossing subdivision

Table 2 – List of locations where we failed to collect Banded Killifish in Illinois, Indiana, and Wisconsin during our 2017-2020 study.

State	County	Stream / Lake	Basin	Date	Common location	Lat / Long	Collectors	Methods	Effort
IL	Lake	Des Plaines River	(Illinois RIver Dr.)	4-Aug-20	Libertyville, East Rockland Road	42.27663, -87.93909	JS Tiemann, K Cook, K Conatser	Seine	30 min
IL	Lake	Des Plaines River	(Illinois RIver Dr.)	4-Aug-20	west of Gurnee	42.34357, -87.94092	JS Tiemann, K Cook, K Conatser	Seine	30 min
IL	Lake	Cedar Lake	(Fox River - Illinois River Dr.)	31-Jul-19	Lake Villa	42.41921, -88.08532	JS Tiemann, AJ Stites, HY Ruellan	Seine	30 min
IL	Lake	Deep Lake	(Fox River - Illinois River Dr.)	31-Jul-19	Lake Villa	42.41668, -88.06451	JS Tiemann, AJ Stites, HY Ruellan	Seine	30 min
WI	Kenosha	Elizabeth Lake	(Fox River - Illinois River Dr.)	17-Jul-19	2 mi E Genoa City	42.50935, -88.27516	JS Tiemann + 2 techs	Seine	30 min
IL	Lake	Gavin Bog	(Fox River - Illinois River Dr.)	18-Jun-20	Ingleside	42.39093, -88.13252	JS Tiemann, K Cook, J Holtswarth	Dipnetting	30 min
IL	Lake	Grays Lake	(Fox River - Illinois River Dr.)	2017 & 2018	Grays Lake	42.33946, -88.04677	JS Tiemann, et al.	Seine	30 min
IL	Lake	Lake Zurich proper	(Fox River - Illinois River Dr.)	28-Aug-19	Lake Zurich	42.20189, -88.11081	JS Tiemann, JL Sherwood, et al.	Seine	30 min
WI	Kenosha	Silver Lake	(Fox River - Illinois River Dr.)	15-Aug-19	Silver Lake	42.55854, -88.14811	JS Tiemann, AJ Stites	Seine	30 min
IL	Lake	Turner Lake	(Fox River - Illinois River Dr.)	27-Aug-19	Chain o' Lakes	42.45238, -88.19073	JS Tiemann, JL Sherwood, et al.	Seine	30 min
IL	Bureau	Hennepin Canal	(Illinois & Mississippi rivers)	18-Sep-19	"The Summit", 14 mi NE of Kewanee	41.40869, -89.77094	JS Tiemann, JL Sherwood	3 mini-fykes set	one night
IL	Bureau	Hennepin Canal	(Illinois & Mississippi rivers)	18-Sep-19	At the Hennepin Canal Parkway park	41.38434, -89.69311	JS Tiemann, JL Sherwood	1 mini-fyke & 4 minnow traps	one night
IL	Kane	Fox River	(Illinois RIver Dr.)	6-Aug-19	Fox River Shores FP	42.12536, -88.28481	JL Sherwood, AJ Stites	Seine	30 min
IL	Kane	Fox River	(Illinois River Dr.)	6-Aug-19	Voyageur Landing FP	42.06781, -88.27192	JL Sherwood, AJ Stites	Seine	30 min
IL	Kane	Fox River	(Illinois River Dr.)	6-Aug-19	Elgin Shores FP	42.01809, -88.27794	JL Sherwood, AJ Stites	Seine	30 min
IL	Kendall	Fox River	(Illinois River Dr.)	28-Jul-19	Yorkville	41.63992, -88.48628	JS Tiemann, K Cook, B Nixon	Boat shocking	60 min
IL	Lake	Fox River	(Illinois River Dr.)	6-Aug-19	Grassy Lake FP, Lake Barrington	42.21961, -88.18203	JL Sherwood, AJ Stites	Seine	30 min
IL	Lake	Fox River	(Illinois River Dr.)	4-Aug-20	Fox Lake	42.40345, -88.18322	JS Tiemann, K Cook, K Conatser	Seine	30 min
IL	LaSalle	Fox River	(Illinois River Dr.)	7-Aug-19	below Dayton Dam	41.38661, -88.78996	JL Sherwood, AJ Stites	Seine	30 min
IL	LaSalle	Vermilion River	(Illinois River Dr.)	28-Jul-19	Oglesby	41.30231, -89.03821	JS Tiemann, K Cook	Seine	30 min
WI	Marinette	Wolf Creek	(Lake Michigan Dr.)	2-Jun-20	~25 mi NW Marinette	45.42091, -87.90513	JS Tiemann, Geoff P.	Seine	30 min
IL	Cass	Illinois River	(Mississippi River Dr.)	8-Jul-20	Beardstown, I-67	40.01688, -90.44646	JS Tiemann, K Cook	Seine	30 min
IL	Marshall	Illinois River	(Mississippi River Dr.)	6-Jul-20	Henry, boat loaunch	41.10985, -89.35201	JS Tiemann, K Cook	Seine	30 min
IL	Henderson	Mississippi River	(Mississippi River Dr.)	13-Jul-20	Oquawka	40.93478, -90.95706	JS Tiemann, K Cook	Seine	30 min
IL	Mercer	Mississippi River	(Mississippi River Dr.)	13-Jul-20	New Boston	41.16901, -91.00311	JS Tiemann, K Cook	Seine	30 min
IL	Mercer	Mississippi River	(Mississippi River Dr.)	13-Jul-20	Keithsberg	41.09797, -90.94426	JS Tiemann, K Cook	Seine	30 min
IA	Muscantine	Mississippi River	(Mississippi River Dr.)	13-Jul-20	Muscatine	41.42013, -91.04341	JS Tiemann, K Cook	Seine	30 min
IA	Muscantine	Mississippi River	(Mississippi River Dr.)	13-Jul-20	Freeport	41.43981, -90.89277	JS Tiemann, K Cook	Seine	30 min
IL	Rock Island	Mississippi River	(Mississippi River Dr.)	14-Jul-20	Rock Island	41.49312, -90.60375	JS Tiemann, K Cook	Seine	60 min
IA	Scott	Mississippi River	(Mississippi River Dr.)	13-Jul-20	Buffalo	41.45321, -90.74271	JS Tiemann, K Cook	Seine	30 min
IL	Whiteside	Coon Creek	(Rock River Dr.)	6-Jul-20	Prophetstown, Star Road	41.67222, -89.91992	JS Tiemann, K Cook	Seine	30 min
IL	Rock Island	Mill Creek	(Rock River Dr.)	13-Jul-20	Milan	41.45461, -90.55983	JS Tiemann, K Cook	Seine	30 min
WI	Jefferson	Rock Lake	(Rock River Dr.)	5-Aug-20	Lake Mills	43.08350, -88.92314	JS Tiemann, K Cook, K Conatser	Seine	30 min
WI	Jefferson	Lake Koshkonong	(Rock River Dr.)	5-Aug-20		42.82941, =89.02144	JS Tiemann, K Cook, K Conatser	Seine	30 min
WI	Waukesha	Goose Lake	(Rock River Dr.)	18-Jun-20	4.5 mi SSE of Oconomowo	43.04783, -88.48028	JS Tiemann, K Cook, J Holtswarth	Seine	30 min
WI	Waukesha	Oconomowoc River	(Rock River Dr.)	18-Jun-20	1 mmi SW of Oconomowo	43.09529, -88.52114	JS Tiemann, K Cook, J Holtswarth	Seine	30 min

Table 3 – The number (#) and percent (%) of collect specimens identified as the native Western subspecies (*F. diaphanus menona*) or non-native Eastern subspecies (*F. diaphanus diaphanus*) based on the mitochondrial DNA D-loop region per April and Turgeon (2006).

State	County	Stream / Lake	Basin	Western	Eastern	Lat / Long
IL	Lake	Bangs Lake	(Fox River Dr.)	10, 100%	0, 0%	42.2689, -88.1375
IL	Lake	Lake Zurich rentention pond	(Fox River Dr.)	9, 100%	0, 0%	42.2037, -88.1101
IL	Lake	Loon Lake	(Fox River Dr.)	3, 100%	0, 0%	42.4511, -88.0783
WI	Kenosha	Cross Lake	(Fox River Dr.)	9, 100%	0, 0%	42.4983, -88.0907
WI	Walworth	Lake Geneva	(Fox River Dr.)	1, 100%	0, 0%	42.5594, -88.4667
IL	Will	Des Plaines River	(Illinois River Dr.)	0, 0%	9, 100%	41.5107, -88.0925
IL	Cook	Chicago Sanitary & Ship Canal	(Lake Michigan - Illinois River Dr.)	0, 0%	10, 100%	41.7927, -87.8168
IL	Cook	North Branch Chicago River	(Lake Michigan - Illinois River Dr.)	0, 0%	5, 100%	41.9160, -87.6619
IL	Will	Chicago Sanitary & Ship Canal	(Lake Michigan - Illinois River Dr.)	0, 0%	9, 100%	41.5906, -88.0674
IL	Cook	Lake Michigan	(Lake Michigan Dr.)	0, 0%	1, 100%	41.7762, -87.5718
IL	Cook	Lake Michigan	(Lake Michigan Dr.)	0, 0%	10, 100%	41.9674, -87.6407
IL	Cook	Wolf Lake - center-west pond	(Lake Michigan Dr.)	1, 10%	9, 90%	41.6691, -87.5359
IL	Cook	Wolf Lake - northeast pond	(Lake Michigan Dr.)	5, 50%	5, 50%	41.6620, -87.5253
IL	Cook	Wolf Lake - southwest pond	(Lake Michigan Dr.)	4, 40%	6, 60%	41.6642, -87.5363
IL	Cook	Powderhorn Lake	(Lake Michigan Dr.)	5, 100%	0, 0%	41.6397, -87.5332
IL	Lake	Lake Michigan	(Lake Michigan Dr.)	0, 0%	5, 100%	42.4924, -87.8030
IL	Lake	Lake Michigan	(Lake Michigan Dr.)	0, 0%	9, 100%	42.3645, -87.8139
IN	Lake	Lake Michigan	(Lake Michigan Dr.)	0, 0%	9, 100%	41.6199, -87.2696
IN	Lake	Wolf Lake	(Lake Michigan Dr.)	0, 0%	1, 100%	41.6682, -87.5116
WI	Racine	Lake Michigan	(Lake Michigan Dr.)	0, 0%	10, 100%	42.7467, -87.7815
WI	Racine	Root River	(Lake Michigan Dr.)	0, 0%	1, 100%	42.7257, -87.7958
IL	Grundy	Illinois River	(Mississippi River Dr.)	0, 0%	9, 100%	41.4016, -88.2800
IL	Grundy	Illinois River	(Mississippi River Dr.)	0, 0%	9, 100%	41.3203, -88.7005
IL	Hancock	Mississippi River	(Mississippi River Dr.)	6, 67%	3, 33%	40.4085, -91.3526
IL	Knox	pond - Snake Den Hollow	(Mississippi River Dr.)	0, 0%	10, 100%	41.0240, -90.0810
IL	La Salle	Illinois River	(Mississippi River Dr.)	0, 0%	4, 100%	41.3269, -89.0061
IL	Rock Island	Rock River	(Mississippi River Dr.)	1, 33%	2, 67%	41.4621, -90.4984
IL	Whiteside	Rock River	(Mississippi River Dr.)	1, 100%	0, 0%	41.6726, -89.9316
IL	Lake	Des Plaines River	(upper Des Plaines River Dr.)	5, 100%	0, 0%	42.3053, -87.9547
IL	Lake	Sanctuary Pond	(upper Des Plaines River Dr.)	10, 100%	0, 0%	42.3338, -88.0135

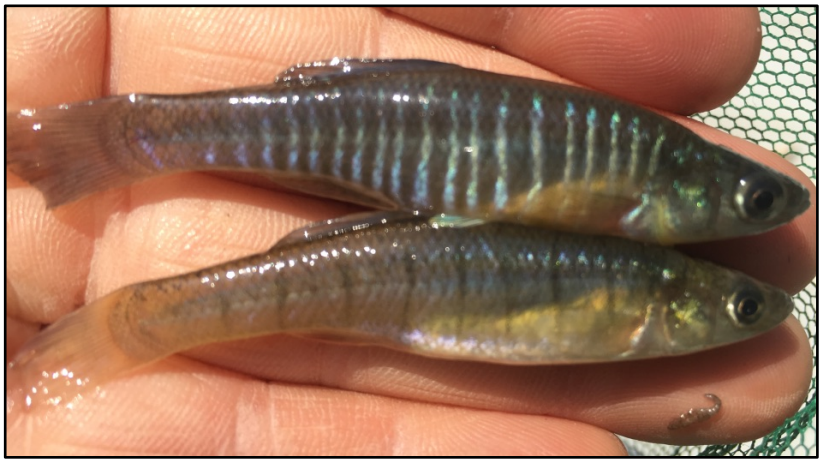
Figure 1 – The Banded Killifish (*Fundulus diaphanus*; photo from the North American Native Fishes Association – www.nanfa.org) and known life history attributes:

The Banded Killifish usually schools near the surface among vegetation in the quiet waters of coastal wetlands, drowned river mouths, and slow-moving streams (Smith 1979). The killifish spawns in late spring and early summer when water temperatures are around 70° F (Becker 1983). Spawning occurs in aquatic vegetation, where the small clusters of eggs adhere to aquatic vegetation and their incubation period can last a couple of weeks (Becker 1983). Anthropogenic disturbances, including extensive residential development of shorelines, high nutrient and siltation loads, and removal of aquatic vegetation, are among the biggest threats to the species (Smith 1965; Trautman 1981; Becker 1983). Specifically within Illinois, the historical range of the Banded Killifish has diminished as a result of the drainage of marshes, sloughs, and natural lakes – factors that have the potential to reduce aquatic vegetation, increase turbidity in streams, support the establishment of non-native species – and continued urbanization of northeastern Illinois (Smith 1971, 1979).

Interestingly, the Banded Killifish was thought to be expanding its range across much of northern Illinois (Rivera et al. 2013; Tiemann et al. 2015; Willink et al. 2018). Since 2000, the Banded Killifish has been collected in large numbers (>100) from several locations in the Chicago metropolitan region. However, the reason for this rapid expansion, including throughout the Chicago Area Waterway System (CAWS) and Mississippi River, appears to be an invasion of the non-native Eastern Banded Killifish (Willink et al. 2018), thus posing another threated– through hybridization or competition/displacement – to the already imperiled taxa. Historical and current distribution of the Western Banded Killifish can be found in Figure 3 and Figure 3.

A)

A)



A)

Figure 2 – (A) Preserved Western Banded Killifish (*Fundulus diaphanus diaphanus*), (B) live Eastern Banded Killifish (*Fundulus diaphanus diaphanus*), and (C) their hybrid preserved (A and C – P. Willink, photo; B – J. Tiemann, photo). Based upon Hubbs and Lagler (1947) and Willink et al. (2018), the Western subspecies has <44 lateral scales, the Eastern subspecies has >48 lateral scales, and their hybrids have an intermediate 44-48.

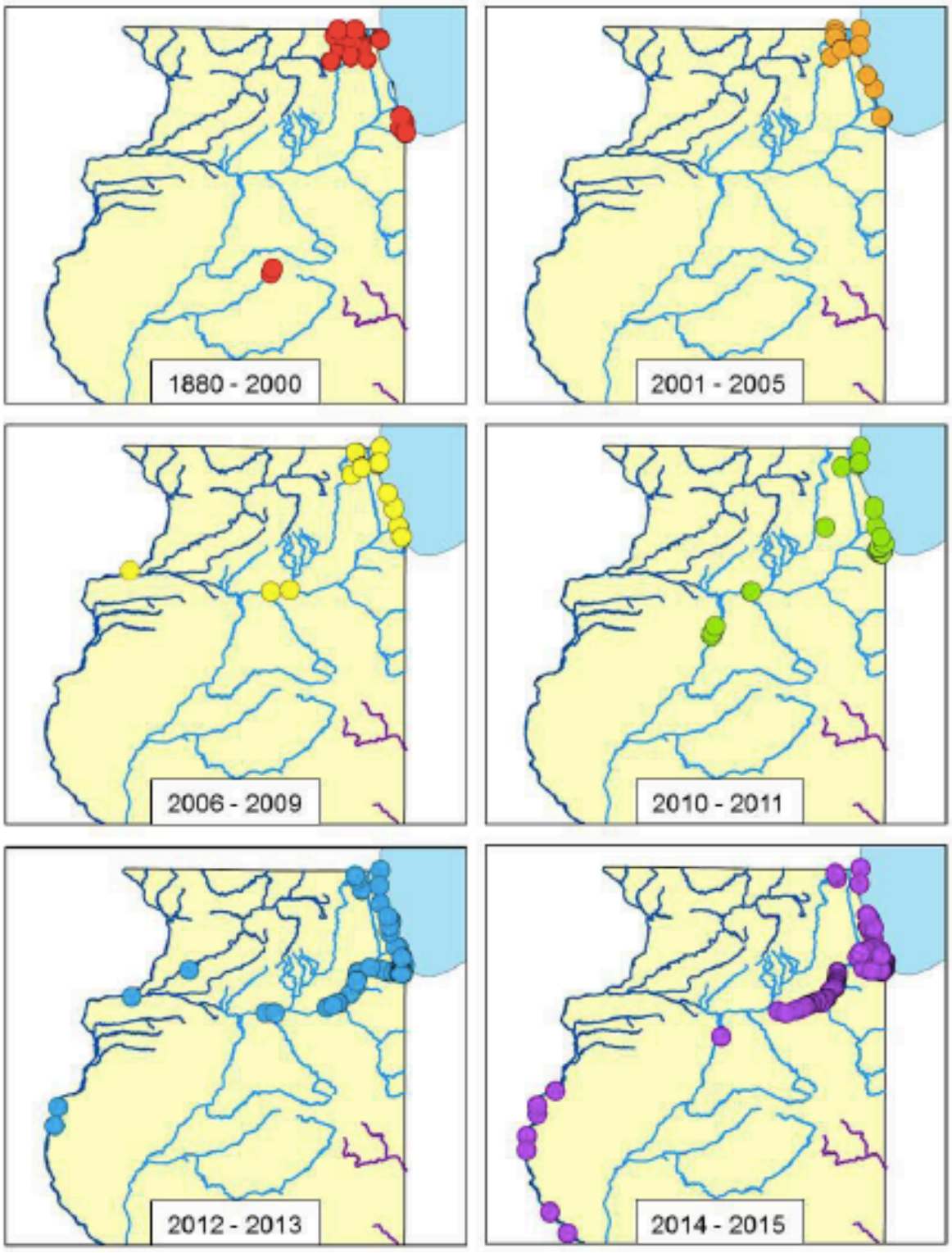


Figure 3 – Distribution of Banded Killifish *Fundulus diaphanus* in Illinois divided into six time periods. The intent of the different periods is to show the changes in distribution over time. (map taken from Willink 2017).

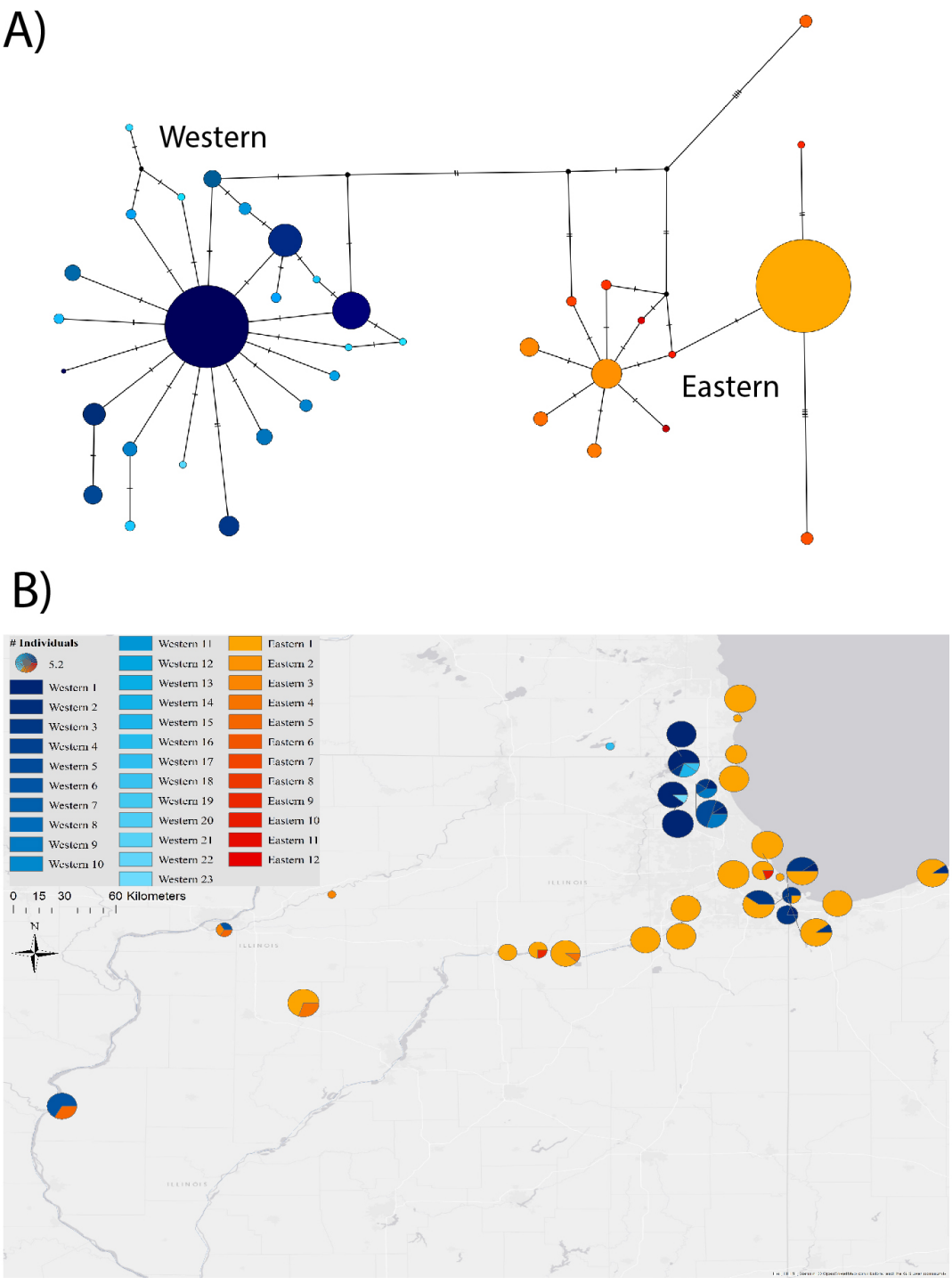


Figure 4 – (A) Banded Killifish haplotype network and (B) distribution by proportion haplotypes within Illinois as georeferenced pie charts. Eastern Banded Killifish are represented by warmer (yellow, orange, red) haplotypes and Western Banded Killifish are represented by cooler (blue) haplotypes. Number of specimens sequenced per site is scaled as pie chart size.

Appendix 1 – Willink, P.W., T.A. Widloe, V.J. Santucci, Jr., D. Makauskas, J.S. Tiemann, S.D. Hertel, J.T. Lamer, and J.L. Sherwood. 2018. Rapid expansion of Banded Killifish *Fundulus diaphanus* across northern Illinois: Dramatic recovery or invasive species? American Midland Naturalist 179:179-190.

Rapid Expansion of Banded Killifish Fundulus diaphanus across Northern Illinois: Dramatic Recovery or Invasive Species?

Author(s): Philip W. Willink, Tristan A. Widloe, Victor J. Santucci Jr., Daniel Makauskas, Jeremy S. Tiemann, Samantha D. Hertel, , James T. Lamer, and Joshua L. Sherwood
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Rapid Expansion of Banded Killifish Fundulus diaphanus across Northern Illinois: Dramatic Recovery or Invasive Species?

Philip W. Willink[1]
Daniel P. Haerther Center for Conservation and Research, Shedd Aquarium, 1200 South Lake Shore Drive, Chicago, Illinois 60605

Tristan A. Widloe[2]
Aquatic Nuisance Species Program, Illinois Department of Natural Resources, 5931 Fox River Drive, Plano 60545

Victor J. Santucci Jr.[3] and Daniel Makauskas[4]
Lake Michigan Program, Illinois Department of Natural Resources, 9511 West Harrison Street, Des Plaines 60016

Jeremy S. Tiemann[5]
Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, 1816 South Oak Street, Champaign 61820

Samantha D. Hertel[6]
Loyola University Chicago, Department of Biology, 1032 West Sheridan Road, Chicago, Illinois 60626

Daniel P. Haerther Center for Conservation and Research, Shedd Aquarium, 1200 South Lake Shore Drive, Chicago, Illinois 60605

James T. Lamer[7]
Department of Biological Sciences, Western Illinois University, 372 Waggoner Hall, 1 University Circle, Macomb 61455

AND

Joshua L. Sherwood[8]
Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, 1816 South Oak Street, Champaign 61820

ABSTRACT.—The distribution of the Illinois state-threatened Banded Killifish *Fundulus diaphanus* has remained largely unchanged in Illinois from 1880 to 2000, being restricted mainly to the northeastern corner of the state. One population identified as Western Banded Killifish *F. d. menona* has remained stable in the glacial lakes region along the southeastern Wisconsin–northeastern Illinois border. Starting in 2001 a second population began to spread and become more common along the Lake Michigan shoreline. From there the population expanded through the Chicago Area Waterway System into the Des Plaines River and eventually

[1] Corresponding author: e-mail: pwillink@sheddaquarium.org
[2] e-mail: Tristan.Widloe@illinois.gov
[3] e-mail: Vic.Santucci@illinois.gov
[4] e-mail: Dan.Makauskas@illinois.gov
[5] e-mail: jtiemann@illinois.edu
[6] e-mail: shertel@luc.edu
[7] e-mail: jt-lamer@wiu.edu
[8] e-mail: jsherwo2@illinois.edu

the Illinois River. Historical museum specimens from this region are identified as *F. d. menona*, but recent specimens are identified as hybrids between *F. d. menona* and Eastern Banded Killifish *F. d. diaphanus*. Subsequently, a third population appeared in the Mississippi River near the mouth of the Rock River in 2009 and has spread from there. These individuals are also identified as *F. d. menona*. The rapid expansion of Banded Killifish from Lake Michigan to the Illinois River appears to be an invasion of the Eastern subspecies *F. d. diaphanus* and the subsequent hybridization with the native Western subspecies. It is unknown where the Banded Killifish in the Mississippi River originated, possibly from populations 160+ kilometers upstream or human introductions. As the Illinois River and Mississippi River populations continue to expand their ranges, their ecological impacts are unknown at this time.

INTRODUCTION

Rapid changes in a species' distribution involving the increasing range of an invasive species (*e.g.*, Irons *et al.*, 2006; Kolar *et al.*, 2007) or the decreasing range of a sensitive species (Metzke *et al.,* 2012) are known to occur. Far less common is the increasing range of a sensitive species, especially when there is no known intentional stocking or translocation by natural resource agencies. Un-aided range expansion of sensitive species raises the hope that perhaps they are responding to improvements in environmental quality. If this is indeed the case, then there is great interest in attempting similar environmental improvements elsewhere to expand the ranges of these sensitive species even further. A situation like this is currently developing in the state of Illinois where Banded Killifish *Fundulus diaphanus* have been listed as state-threatened since 1989 (Mankowski, 2012).

The species as a whole is considered secure over its entire range, which extends from the Dakotas eastward through the Great Lakes to Newfoundland, and then south along the Atlantic coast to South Carolina (Scott and Crossman, 1973; Page and Burr, 2011). The eastern half of the range is generally considered to be inhabited by the subspecies Eastern Banded Killifish *Fundulus diaphanus diaphanus*, whereas the western half is inhabited by the subspecies Western Banded Killifish *Fundulus diaphanus menona* (Hubbs and Lagler, 1947; based on Shapiro, 1947). Genetic divergence between the two subspecies, according to mitochondrial D-Loop, is 2% and dates the split between subspecies to approximately 570,000 y ago (April and Turgeon, 2006). Introgression between the two subspecies is known (April and Turgeon, 2006), as well as hybridization with other species, such as the Mummichog *Fundulus heteroclitus* (Weed, 1921; Hubbs *et al.,* 1943; Griffith, 1968, 1972; Fritz and Garside, 1974; Dawley, 1992; Dawley *et al.*, 1999, 2000; Hernández Chávez and Turgeon, 2007; Mérette *et al.*, 2009).

Illinois is along the southern edge of the Western Banded Killifish range, with historical records primarily in northeast Illinois and two in the center of the state (Forbes and Richardson, 1919, 1920; Smith, 1979). Fish biologists in recent years are collecting more Banded Killifish and from locations where they have not previously been recorded. The goals of this manuscript are to (1) document changes in the distribution of Banded Killifish in Illinois from 1880 to the present and (2) assess reasons that may be driving these distributional changes. This information is critical for the successful management of Banded Killifish that is designated a Species in Greatest Conservation Need (*e.g.*, state-threatened) and whose recovery is a goal of the Illinois Wildlife Action Plan (Illinois Department of Natural Resources, 2016).

MATERIALS AND METHODS

Locality data were based primarily on vouchered specimens in fish collections at The Field Museum (FMNH), Chicago and the Illinois Natural History Survey (INHS), Champaign

[museum acronyms based on Sabaj (2016)]. Included with these records were significant amounts of field observation data from the Illinois Department of Natural Resources–Aquatic Nuisance Species Program conducting surveys in Illinois rivers. The Illinois Department of Natural Resources–Lake Michigan Program provided field observation data from five annual beach surveys (south to north; Jackson–Outer Harbor, Farwell Avenue, Tower Road, Waukegan, and North Point) starting in 1979. Supplemental data included personal observations from several of the authors and colleagues, as well as literature records that are considered reliable (*e.g.*, Meek and Hildebrand, 1910; Rivera *et al.,* 2013; Tiemann *et al.*, 2015; Hrabik, 2016; Lamer *et al.,* 2016; Schmidt, 2016).

These types of data are considered 'presence only' and do not explicitly take into consideration absences from locations where Banded Killifish are historically known to occur. However, some of the data sources, such as the Illinois Department of Natural Resources–Lake Michigan Program annual beach surveys and the Long-Term Illinois River Fish Population Monitoring Program (also known as Long Term Electrofishing), do include repeated surveys at the same sites over long time periods. In other instances there are examples of areas that were the focus of short-term intensive surveys, such as Will County (Willink and Veraldi, 2009) and Lake Calumet (Greenfield and Rogner, 1984). Survey methods, effort, and reporting have also changed over the past 130+ y. There will always be gaps in our understanding, but based on the considerable number of fish surveys that have been conducted in Illinois, we believe the general changes in distribution patterns of Banded Killifish in Illinois are real and not simply artifacts of sampling error, changes in methodology, or increased sampling effort. Because fish survey methods have changed considerably over the years, it was not possible to determine a standardized catch per unit effort with this data.

Identification of preserved specimens in the collections of the FMNH and INHS was based on lateral row scales, which were counted according to Hubbs and Lagler (1947). Although the character is variable, it has been demonstrated to be informative over the entire geographic range of the species (Shapiro, 1947, in Hubbs and Lagler, 1947; April and Turgeon, 2006; Hernández Chávez and Turgeon, 2007). Other characters, such as fin ray counts and pigmentation, were explored. However, normal fin ray counts between these subspecies differ on average by one fin ray, with considerable overlap in the range of counts between them. Pigmentation is difficult due to changes during ontogeny.

Data for specimens identified as the Western subspecies *F. d. menona* from Minnesota, Iowa, South Dakota, Illinois, and Indiana were combined because of considerable overlap of lateral row scale counts among regions. These values were consistent with what has previously been reported in the literature (Shapiro, 1947, in Hubbs and Lagler, 1947). All museum specimens examined were collected prior to 1957, well before any purported changes in distribution patterns.

Specimen data for individuals identified as the Eastern subspecies *F. d. diaphanus* were based on specimens from Maine as well as mean lateral row scale values reported from Hernández Chávez and Turgeon (2007). The literature values were included because only a few museum specimens of *F. d. diaphanus* were readily available, and Hernández Chávez and Turgeon (2007) corroborated their results with genetic data.

RESULTS

DISTRIBUTION

The distribution of Banded Killifish in Illinois is fairly consistent from 1880–2000 (Fig. 1). There are two records from the center of the state, but both date to 1880. They have not

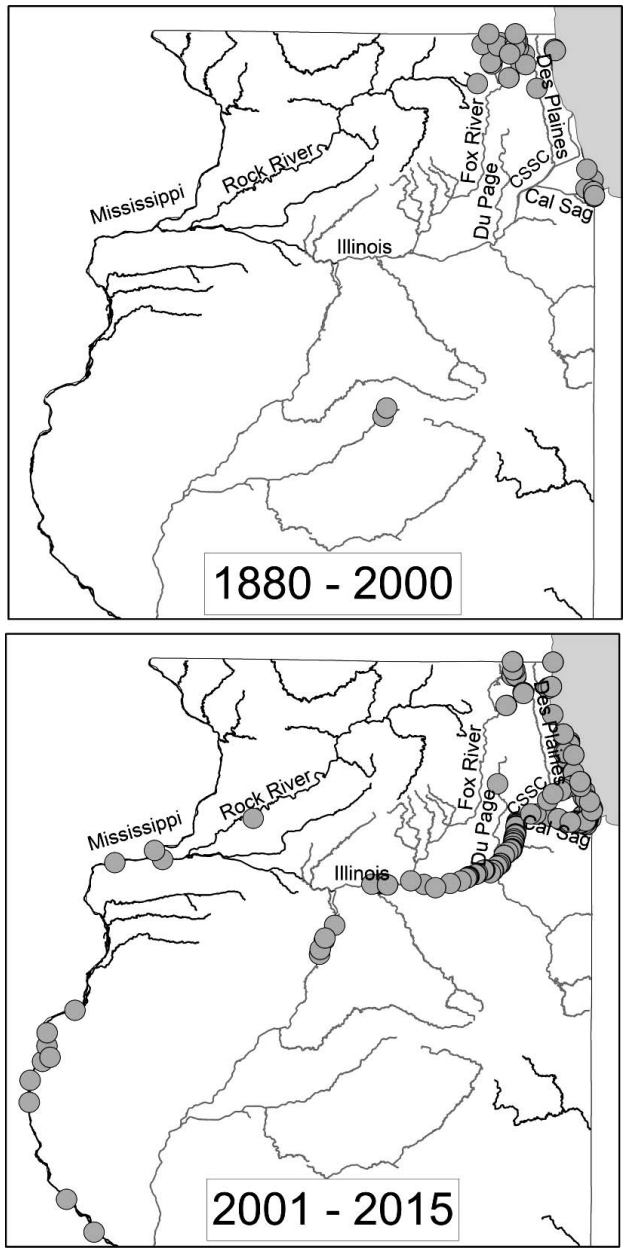

FIG. 1.—Distribution of Banded Killifish *Fundulus diaphanus* in Illinois divided into two time periods

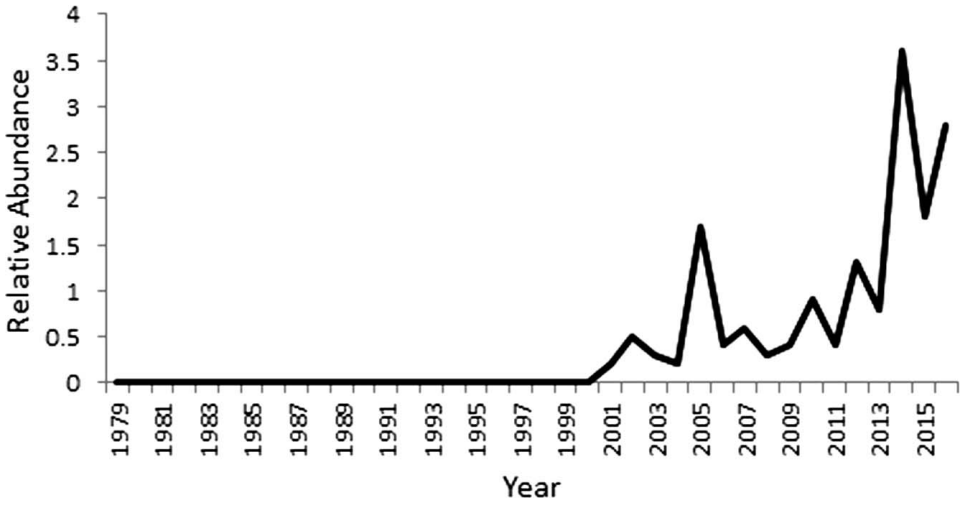

FIG. 2.—Relative abundance (mean number of fish/seine haul) of Banded Killifish *Fundulus diaphanus* based on beach seine sampling at five locations along the Lake Michigan shoreline in Illinois, 1979–2016

been reported from the Sangamon watershed since and are considered extirpated (Smith, 1979). The rest of the records are restricted to northeastern Illinois, with population centers in the glacial lakes region near the Wisconsin border and the Calumet region adjacent to Indiana (Forbes and Richardson, 1919, 1920; Smith, 1979; Retzer and Batten, 2005; Willink, 2009).

Banded Killifish were common in the mouths of Lake Michigan tributaries in the late 1800s (Nelson, 1876; Jordan, 1878) but disappeared through most of the 20th century (Smith, 1979). However, they began appearing in standardized Illinois Department of Natural Resources Lake Michigan Program annual beach surveys in 2001 (Fig. 2). They have been present ever since and catch rates have increased over time. Locations are scattered along the entire length of the Lake Michigan shoreline in Illinois (Fig. 1).

Although Banded Killifish were known to occur in the Calumet Region since record keeping started, this population appeared to increase in this area around 2010. From there they spread down the Calumet Sag Channel, the Chicago Sanitary and Shipping Canal, the Des Plaines River, and into the upper Illinois River (Fig. 1). There is no evidence (*e.g.,* from Illinois Department of Natural Resources routine basin surveys) for Banded Killifish from the Illinois or lower Des Plaines rivers moving up the Fox or Des Plaines rivers to the glacial lakes along the Wisconsin border. As a side note, the Forest Preserve District of DuPage County transplanted Banded Killifish from northern Illinois to a site along the West Branch of the DuPage River in 2010, but the fish have not been seen since at the site.

The first record for Banded Killifish in the Illinois River was 2006 near Starved Rock State Park in Utica, LaSalle County (Fig. 1). Around 2010 the population expanded downstream to the Marshall–Peoria County line. From 2001 to 2010, the average rate of range expansion of Banded Killifish from Lake Michigan to the Marshall–Peoria County line on the Illinois River was 25.4 river km/y.

Conversely, the first record for Banded Killifish along the Illinois border of the Mississippi River was in 2009 in Davenport, Iowa, (John Olson, Ben Hucka, and Jerad Stricker, Iowa Department of Natural Resources, pers. comm.), which is opposite Rock

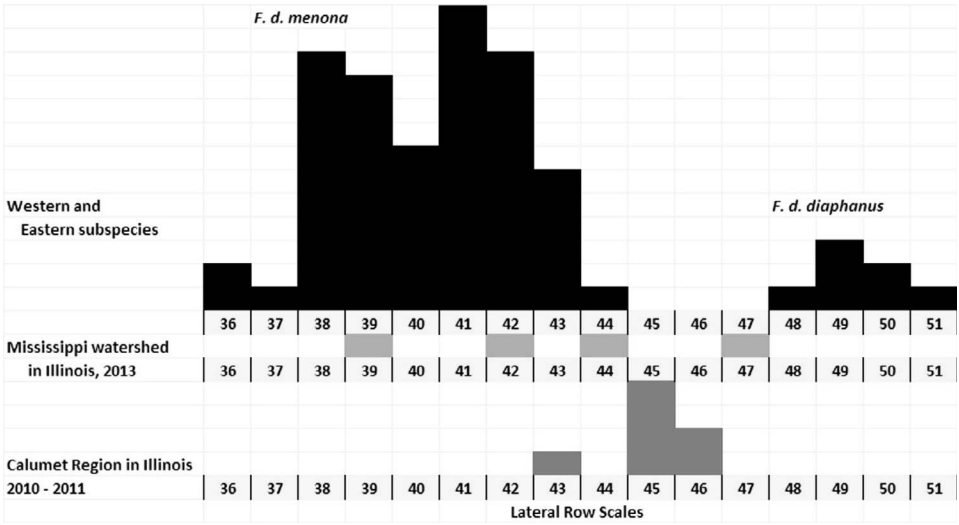

FIG. 3.—Lateral row scales of Banded Killifish *Fundulus diaphanus* from different regions and different time periods. Sample sizes are *F. d. menona* (N = 62), *F. d. diaphanus* (N = 7), Mississippi watershed (N = 4), and Calumet Region (N = 7)

Island County, Illinois (Fig. 1). Around 2010 the Mississippi River population expanded downstream and moderately up the Rock River. From 2006 to 2015, the average rate of expansion down the Mississippi River from Davenport, Iowa to West Alton, Missouri, was 55.1 river km/y.

Subspecies and hybrid identification.—Lateral row scales for Western Banded Killifish, including specimens from Illinois collected prior to 1957, ranged from 36 to 44, with all but one value between 38–43 (n = 62) (Fig. 3). Eastern Banded Killifish had 48–51 lateral row scales (n = 7).

Specimens collected in the Calumet Region in 2010 and 2011 had 43 to 46 lateral row scales, with six of the seven vouchers with either 45 or 46 lateral row scales (n = 7) (Fig. 3). These values are intermediate between the Western and Eastern values. Hybrids between Western Banded Killifish and Eastern Banded Killifish exhibit a similar intermediate pattern elsewhere (Shapiro, 1947, in Hubbs and Lagler, 1947; April and Turgeon, 2006; Hernández Chávez and Turgeon, 2007); therefore, we are tentatively identifying the recent Calumet individuals as hybrids between the Western and Eastern subspecies. Specimens collected in the Calumet Region prior to 1937 had 39–44 lateral row scales with a mean of 42 (n = 23).

Specimens collected in the Mississippi River proper in 2013 had 39 and 44 lateral row scales, which identify them as Western Banded Killifish (n = 2). Specimens collected in Coon Creek (the most upstream Rock River locality) in 2013 had 42 and 47 lateral row scales (n = 2). The individual with 42 scales is consistent with the other Western Banded Killifish identified from the Mississippi watershed. The individual with 47 lateral row scales is difficult to identify because it is just below values for Eastern Banded Killifish, and conceivably within the range of hybrids.

DISCUSSION

Populations in the distribution of Banded Killifish remained unchanged for over a century (Smith, 1979). Most glacial lakes populations appeared stable, although there may have been a decline in the Fox Chain O'Lakes, which is a subset of lakes that the Fox River flows directly through that has been heavily impacted by development. Known populations in Wolf Lake (Willink, 2009) and Powder Horn Lake (Retzer and Batten, 2005) in the Calumet Region also have persisted over time.

Banded Killifish abundance increased along the shoreline of Lake Michigan at the beginning of the 21st century and eventually cascaded down the Illinois River. Environmentally, the Great Lakes are continually exposed to different conditions and stressors, with introduced species being a common item of concern. Invasive Zebra Mussels *Dreissena polymorpha* and Quagga Mussels *Dreissena rostriformis bugensis* became established in the 1980s and 1990s (Nalepa *et al.,* 2001), filtering plankton from the water column, therefore increasing water clarity. A consequence of increased water clarity is increased macrophyte growth. This benefits Banded Killifish because they use vegetation to spawn (Richardson, 1939). Concordant with the increase in mussel numbers was a decrease in invasive Alewife *Alosa pseudoharengus* (Bunnell *et al.,* 2015; Warner *et al.,* 2015), which are known to have negative impacts on numerous Lake Michigan fish species via predation on larvae and smaller individuals (Madenjian *et al.*, 2008).

Physical aspects of Lake Michigan are shifting as well. Water temperature, for example, is increasing with climate change, with a particularly large jump of about 2.5 C during the 1997–1998 El Niño event (Gronewold and Stow, 2014). Pollution is on the decline [*e.g.,* drops in PCB levels in salmon (Rasmussen *et al.,* 2014)] attributable to decades of increased environmental regulations, although pollution is certainly still present in the region. Similarly, the upper Illinois River was so heavily polluted it became devoid of freshwater mussels (Starrett, 1971). However, after the passage of the Clean Water Act, conditions improved and mussels recolonized the area (Sietman *et al.*, 2001).

As to whether any of these biological or environmental factors, an unknown factor(s), or combination of factors played a role in the expansion of Banded Killifish in Illinois is unclear. Several could have improved environmental conditions for Banded Killifish and facilitated population increases, even if they were not the root cause of the increase. One reason it is difficult to determine which factors may have contributed to the range expansion is because many of the Banded Killifish in Illinois today do not appear to be the same subspecies of Banded Killifish present in Illinois in the past.

Prior to 1989 Banded Killifish in Illinois appeared to belong to the Western subspecies. Recent records (1989–today) are sparse because it is listed as state-threatened in Illinois, hence collection of vouchers is strongly discouraged and regulated by permits. The few specimens that have been preserved recently in museum collections have lateral row scale counts intermediate between the Western subspecies and the Eastern subspecies. This is a common introgression pattern among fishes, with hybrids often intermediate between the two parent species (Hubbs, 1955).

Hybrids have been documented between Banded Killifish subspecies. Hubbs and Lagler (1947) based on data from Shapiro (1947) reported an intergrade zone between the two subspecies in Lake Ontario and the St. Lawrence River. The thought was the subspecies occupied separate refugia during the last glaciation, with the Eastern subspecies occupying an area along the Atlantic coast and the Western subspecies in the Mississippi River (Bailey and Smith, 1981). As the glaciers retreated, each subspecies expanded their respective ranges until they made secondary contact and introgressed. Genetic patterns among Banded

Killifish populations along the St. Lawrence are consistent with this history (Rey and Turgeon, 2007). This theory was validated by April and Turgeon (2006) who analyzed genetics and morphology. However, they found the 'hybrid zone' to extend into Ohio along Lake Erie, farther than people realized. The western edge of this 'hybrid zone' was identified by mitochondrial DNA from the Eastern subspecies introgressing into the Western subspecies. Farther east, the morphology and nuclear DNA were intermediate between parent subspecies.

Part of the explanation for the biogeographic patterns evident in Illinois today may be that Eastern Banded Killifish or hybrids somehow made it to southern Lake Michigan and spread from there. This is consistent with identifications based on lateral row scales and the westward expansion pattern documented by April and Turgeon (2006) in the eastern Great Lakes. As to why these killifish are expanding their range now, when the Western Banded Killifish previously present in Illinois did not, is unclear. Trautman (1981) reported Eastern Banded Killifish appeared to be more tolerant of pollution and were not as reliant on vegetation for spawning; therefore, it may be that they are better suited to the prevalent habitats in the region than are the original Western Banded Killifish. The native range of Eastern Banded Killifish also extends farther south than that of the Western Banded Killifish. It may be that increasing water temperatures due to climate change are also benefiting these hybrids if Eastern Banded Killifish tolerances are expressed (Hayhoe *et al.*, 2010; Gronewold and Stow, 2014).

These scenarios may explain the increase in the Lake Michigan-Illinois River population and the relative stasis of the glacial lakes population, but they do not explain the appearance and subsequent expansion of the Mississippi River population. There were pre-existing Banded Killifish populations in the Mississippi watershed, notably central Wisconsin, Minnesota, and northwestern Iowa (Becker, 1983; Page and Burr, 2011). Each of these is 160+ km away from the Illinois records in the Mississippi. The largest Illinois population appears to be in Pool 19. This impoundment is heavily vegetated with very low flow (Tazik *et al.,* 1993), acting more like a lake than a river. This is consistent with the habitat preferences of the species. Although, it is possible there was a downstream colonization event from Wisconsin, Minnesota, or Iowa, perhaps associated with recent flooding, we have no intervening records between these upstream populations and the recent Illinois population to support this scenario.

There is an aquatic connection between the Illinois River watershed and the Mississippi watershed via the Hennepin Canal. However, the canal has been defunct for years and it would have been difficult for the fishes to move through the multiple closed locks. Furthermore, the Lake Michigan–Illinois River individuals are putative hybrids, whereas most of the Mississippi River individuals appear to be Western Banded Killifish.

Another possible explanation for the Mississippi River population is an accidental or intentional release by someone. There are documented occurrences of people moving killifish between waterbodies [*e.g.,* western Pennsylvania (Raney, 1938) and in Newfoundland (Mitchell and Purchase, 2014)] but no specific evidence surrounding this possibility for Banded Killifish in the Midwest.

There is still a considerable amount of ambiguity surrounding this rapidly changing range of Banded Killifish in Illinois. Genetic data are required to develop a clearer picture. Due to the potential for directional introgression, mitochondrial and nuclear markers would be necessary. It would even be beneficial to incorporate microsatellites to analyze finer biogeographic patterns within this rapid range expansion, as well as to determine potential source populations.

Nonetheless, there are already important management implications based on the findings reported in this publication. First, in Illinois the listing of a species as state-threatened or state-endangered is decided at the species level. Subspecies are not considered as separate taxonomic units. Hence it does not matter if the Banded Killifish in Illinois are of the Western subspecies, Eastern subspecies, or hybrids. They are all considered equally in determining listing status.

Second, there appear to be at least three management units, each with its particular history, requirements, and potential future trajectories. One is the Lake Michigan to Illinois River population that is made up of putative hybrids. This population could conceivably be divided into sub-populations (*e.g.*, Illinois River, Lake Michigan, etc.). The second is the recent Mississippi River population that continues to expand. The third is the glacial lakes population. There is no indication the rapid expansion occurring elsewhere in the state has reached this region along the Wisconsin border. Expansion here would require movement of fish past dams in the Fox and Des Plaines rivers, as well as their tributaries. Additionally, the connections that occurred between glacial lakes and streams of this area have been disrupted due to development. For the time being, this area appears to be isolated and may be the last remaining original stock of Western Banded Killifish in the state.

One implication of there being different Banded Killifish management units involves stocking considerations. There have been two recent introductions of Banded Killifish authorized by the Illinois Department of Natural Resources–Prairie Crossing in 1998 and the West Branch DuPage River in 2010 mentioned above. Each involved stock populations from glacial lakes, and both of the introduced populations were close to the source populations in northeast Illinois. The Prairie Crossing appears to have worked (Schaeffer *et al.*, 2012; Bland, 2013), whereas there is no evidence to date that the West Branch DuPage River has worked. In regards to future translocations and to preserve original genetics, there needs to be increased scrutiny regarding source populations and stocking locations.

A third management issue is the potential ecological impacts, especially in areas where Banded Killifish had not been found in the past. For example further expansion throughout the Mississippi River system could potentially lead to "unnatural" competitive pressures on species that have not encountered this killifish before. Close monitoring of the expansion is needed.

Determining whether the range expansion represents dramatic recovery or an invasive species is more complicated than originally realized, and it depends upon the taxonomic level used to address the question. At the species level, Banded Killifish as a whole was stable in Illinois, possibly declining slowly, until recent events. From that perspective this is a dramatic recovery. At the subspecies level, if Illinois is on the forefront of the westward expansion of the Eastern Banded Killifish, then it could be considered an invasion, as there are no historical records for Banded Killifish for the Illinois portion of the Mississippi River. This could also be considered an invasion if human intervention is involved, even though this population appears to be from regional stock.

Given current trends, Banded Killifish may continue to expand its range. This expansion could incorporate other states in the region, most notably Indiana, Wisconsin, Iowa, and Missouri. A larger regional view will be needed to determine where the various taxonomic subunits originated and where they may be spreading. Although Banded Killifish historically split into two subspecies that were for a while evolving along their own independent trajectories to potentially becoming full distinct species in the future, they may now be gradually recombining back into a single species whose distribution could be continuing to expand.

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